REPORT

Study Title

MICRONUCLEUS TEST IN BONE MARROW CELLS OF THE MOUSE WITH [redacted]

Author

[redacted]

Study completion date

11 January 2007

Test Facility

NOTOX B.V.
Hambakenwetering 7
5231 DD 's-Hertogenbosch
The Netherlands

Laboratory Project Identification

NOTOX Project [redacted]
NOTOX Substance [redacted]

1. CONTENTS

2. STATEMENT OF GLP COMPLIANCE

NOTOX B.V., 's-Hertogenbosch, The Netherlands

The study described in this report has been correctly reported and was conducted in compliance with:

The Organization for Economic Cooperation and Development (OECD) Good Laboratory Practice Guidelines (1997).

Which essentially conform to:

The United States Food and Drug Administration Good Laboratory Practice Regulations.

The United States Environmental Protection Agency Good Laboratory Practice Regulations.

The sponsor is responsible for Good Laboratory Practice (GLP) compliance for all test substance information unless determined by NOTOX.

Analysis of stability, homogeneity and concentration of the test substance under test conditions was not performed as part of this study.

NOTOX B.V.

[redacted]
Study Director

[redacted]
Head of In Vitro & Environmental Toxicology

[redacted]

Date: 11-01-2007 Date: 11/01/2007

3. QUALITY ASSURANCE STATEMENT

NOTOX B.V., 's-Hertogenbosch, The Netherlands

This report was inspected by the NOTOX Quality Assurance Unit to confirm that the methods and results accurately and completely reflect the raw data.

The dates of Quality Assurance inspections are given below.
During the on-site process inspections procedures applicable to this type of study were inspected.

The reporting date is the date of reporting to the Study Director. The QAU report was then forwarded to the Test Facility Management.

Type of inspections	Phase/Process	Start Inspection date	End Inspection date	Reporting date
Study	Protocol	04-Oct-06	04-Oct-06	04-Oct-06
	Amendment 1 of protocol	03-Nov-06	03-Nov-06	03-Nov-06
	Report	19-Dec-06	19-Dec-06	19-Dec-06
Process	**Genetic and In Vitro Toxicology** Test substance handling Exposure Observations/Measurements Specimen handling	16-Oct-06	20-Oct-06	24-Oct-06
	SPF unit Test substance handling Exposure Observations/Measurements Specimen handling	07-Nov-06	13-Nov-06	21-Nov-06

Head of Quality Assurance
[redacted]

[redacted]

Date: [redacted]

4. SUMMARY

Micronucleus test in bone marrow cells of the mouse with [redacted]

[redacted] was tested in the Micronucleus Test in mice, to evaluate its genotoxic effect on erythrocytes in bone marrow.

The study procedures described in this report were based on the most recent OECD and EEC guidelines.

Batch 0619201 of [redacted] was a dark purple powder with a purity of >95% (NMR). The test substance was suspended in corn oil.

Five male and five female animals were used in each of the six treatment groups, including negative and positive controls. All groups received a single intraperitoneal injection. The negative and positive control groups were treated with vehicle and 50 mg/kg body weight of cyclophosphamide (CP), respectively. Animals were dosed with [redacted] at 15 (two groups), 7.5 (one group), and 3.8 (one group) mg/kg body weight. All animals dosed with 15 mg/kg body weight showed the following toxic signs: lethargy and a hunched posture. Nine male and two female animals had a rough coat after dosing. Five male and four female animals had their eyes closed and two male animals also showed ataxia after dosing. The animals of the dose level of 7.5 and 3.8 mg/kg body weight and the control animals showed no abnormalities after dosing.

Bone marrow of the groups treated with [redacted] was sampled 24 or 48 (highest dose only) hours after dosing. Bone marrow of the negative and positive control groups was harvested 24 and 48 hours after dosing, respectively.

[redacted] induced a statistically significant increase in the mean number of micronucleated polychromatic erythrocytes compared to the corresponding solvent control group in female animals dosed with 7.5 mg/kg b.w. Since the mean number of micronucleated polychromatic erythrocytes per 2000 polychromatic erythrocytes (1.2 ± 1.1) of this group as well as the individual number of micronucleated polychromatic erythrocytes per 2000 polychromatic erythrocytes per animal were well within the historical control data range (0 – 4), the increase was considered not to be biologically relevant.

No increase in the frequency of micronucleated polychromatic erythrocytes, compared to the vehicle treated animals, was observed in the polychromatic erythrocytes of the bone marrow of all other dose groups treated with [redacted]

The incidence of micronucleated polychromatic erythrocytes in the bone marrow of all negative control animals was within the historical solvent control data range. Cyclophosphamide, the positive control substance, induced a statistically significant increase in the number of micronucleated polychromatic erythrocytes in both sexes. Hence, both criteria for an acceptable assay were met.

The groups that were treated with 7.5 and 3.8 mg [redacted] kg b.w. showed no decrease in the ratio of polychromatic to normochromatic erythrocytes compared to the vehicle controls, which reflects a lack of toxic effects of this compound on the erythropoiesis. In the group treated with 15 mg [redacted] kg b.w., sampled after 48 hours, a decrease in the ratio of polychromatic to normochromatic erythrocytes was observed compared to the vehicle controls. This provides evidence that [redacted] reaches the bone marrow and has a toxic effect on the erythropoiesis following a 48 h exposure. The animals treated with 15 mg [redacted] kg b.w. sampled after 24 hours did not show a decrease in the ratio of polychromatic to normochromatic erythrocytes compared to the vehicle controls.

The groups that were treated with cyclophosphamide showed an expected decrease in the ratio of polychromatic to normochromatic erythrocytes compared to the vehicle controls, demonstrating toxic effects on erythropoiesis.

It is concluded that [redacted] is not clastogenic in the micronucleus test under the experimental conditions described in this report.

5. INTRODUCTION

5.1. Preface

Sponsor	[redacted]
Study Monitor	[redacted]
Test Facility	NOTOX B.V. Hambakenwetering 7 5231 DD 's-Hertogenbosch The Netherlands
Study Director	[redacted]
Technical Coordinator	[redacted]
Coordinating Biotechnician	[redacted]
Study Plan	Start : 17 October 2006 Completion : 11 December 2006

5.2. Aims of the study

The purpose of the study was to obtain information on the clastogenicity of [redacted] when administered to mice at a maximum tolerated acute dose, by measuring the increase in the number of micronucleated polychromatic erythrocytes per 2000 polychromatic erythrocytes in mouse bone marrow.

The NMRI BR mouse is used as the test system because it is a readily available rodent species, which is commonly used for this purpose, with documented susceptibility to a wide range of toxic substances.

Background of the test system
The micronucleus test is a mammalian *in vivo* cytogenetic test, which detects damage to the chromosome or to the mitotic apparatus induced by a test substance (1-2).
Basis for this test is the increase in the number of micronucleated polychromatic erythrocytes in the bone marrow of the femora of mice exposed to the test compound compared with control animals. Micronuclei are small particles consisting of acentric fragments of chromosomes or entire chromosomes, which lag behind at anaphase stage of cell division. After telophase, these fragments may not be included in the nuclei of daughter cells and form single or multiple micronuclei in the cytoplasm. When an erythroblast develops into an erythrocyte, the main nucleus is extruded and may leave micronuclei in the cytoplasm. Visualization of micronuclei is facilitated in polychromatic cells because they lack a nucleus. Polychromatic erythrocytes can be distinguished from normochromatic cells by their bluish colour after staining.

A test article, which induces a positive result in this assay, is presumed to be a potential clastogenic agent.

5.3. Guidelines

The protocol was reviewed and agreed by the Laboratory Animal Welfare Officer and the Ethical Committee of NOTOX (DEC NOTOX 98-50) as required by the Dutch Act on Animal Experimentation (February 1997).

The study procedures described in this report were based on the following guidelines:

- European Economic Community (EEC). Directive 2000/32/EC, Part B: Methods for the Determination of Toxicity; B.12: "Mutagenicity: *In Vivo* Mammalian Erythrocyte Micronucleus Test" (published June 8, 2000).
- Organisation for Economic Co-operation and Development (OECD), OECD Guidelines for the Testing of Chemicals, Guideline No. 474: Mammalian Erythrocyte Micronucleus Test (adopted July 21, 1997).

5.4. Storage and retention of records and materials

Records and materials pertaining to the study including protocol, raw data, specimens and the final report are retained in the NOTOX archives for a period of at least 10 years after finalization of the report. After this period, the sponsor will be contacted to determine whether raw data and specimens should be returned to them, retained or destroyed on their behalf.

NOTOX will retain a test substance sample until the expiry date, but no longer than 10 years after finalization of the report. After this period the sample will be destroyed.

6. MATERIALS AND METHODS

6.1. Test substance

6.1.1. Test substance information

Identification	[redacted]
Structure	
Molecular formula	[redacted]
Molecular weight	1317.45
Description	Dark purple powder
Batch	[redacted]
Purity	>95% (NMR)
Test substance storage	At room temperature in the dark
Stability under storage conditions	Stable
Expiry date	01 January 2008

6.1.2. Study specific test substance information

Stability in vehicle	Corn oil: not indicated
Solubility in vehicle	Corn oil: not indicated

6.1.3. Test substance preparation

[redacted] was suspended in corn oil (Roth, Karlsruhe, Germany). [redacted] concentrations were blended and treated with ultra-sonic waves to obtain a homogeneous suspension. [redacted] concentrations were dosed within 3 hours after preparation.

6.2. Reference substances

6.2.1. Negative control

Negative control animals, treated with corn oil.

6.2.2. Positive control

The positive control used in the micronucleus test was cyclophosphamide (CP; CAS no. 50-18-0; Endoxan, Asta-Werke, Germany) dissolved in physiological saline (Ziekenhuis Apotheek Noordoost-Brabant, Den Bosch, The Netherlands) dosed as a single intraperitoneal injection of 50 mg/kg body weight.

The route and frequency of administration and the volume administered of the negative and positive control were the same as those of the test article.

6.3. Test system

NMRI BR mice (SPF) were used as the test system. These mice are recommended by international guidelines (e.g. OECD, EEC). Females were nulliparous and non-pregnant. The animals were provided by Charles River, Sulzfeld, Germany.

In the micronucleus main test 5 male and 5 female mice were treated per sampling time in each treatment group. Young adult animals were selected (6-8 weeks old).

The body weights of the mice at the start of the treatment were within 20% of the sex mean. The mice were identified by a unique number on the tail written with a marker pen. The animals were allocated to treatment groups as they came to hand from the delivery boxes.

On arrival and at the start of the treatment, all animals were clinically examined to ensure selected animals were in a good state of health.

6.4. Animal husbandry

Conditions
The animals were housed in an air-conditioned room with approximately 15 air changes per hour and a controlled environment with a temperature of 21 ± 3°C (actual range 20.1 – 22.6°C) and a relative humidity of 30 – 70% (actual range 40 – 89%). Due to cleaning procedures or performance of functional observations in the room, temporary deviations from the maximum level for humidity (with max. 19%) occurred. Based on laboratory historical data these deviations are considered not to affect the study integrity. The room was illuminated with 12 hours artificial fluorescent light and 12 hours dark per day.

Accommodation
The animals were group housed (5 animals per sex per cage) in labelled polycarbonate cages (type MII height: 14 cm) containing sterilised sawdust as bedding material (Litalabo; S.P.P.S., Argenteuil, France). Paper bedding was provided as nest material (Enviro-dri, TecniLab-BMI BV, Someren, The Netherlands). Certificates of analysis of bedding and paper were examined and then retained in the NOTOX archives. The acclimatisation period was at least 5 days before start of treatment under laboratory conditions.

Diet
The animals had free access to standard pelleted laboratory animal diet (SM R/M-Z from SSNIFF® Spezialdiäten GmbH, Soest, Germany). Each batch was analysed for nutrients and contaminants on a regular basis. Certificates of analysis were examined and then retained in the NOTOX archives.

Water
The animals had free access to tap-water. Certificates of analysis (performed quarterly) were examined and then retained in the NOTOX archives.

6.5. Treatment

The mice received an intraperitoneal injection of a maximum tolerated (high), an intermediate and a low dose of [redacted] The route of administration was chosen to maximize the chance of the test article reaching the target tissue.

The dosing volume was 10 ml/kg body weight.

6.6. Observations

The systemic toxic signs were recorded at least once a day. The time of death was recorded as precisely as possible. The animals were weighed just prior to dosing.

6.7. Study design

6.7.1. Dose range finding study

Selection of an adequate dose range for the micronucleus main test was based on a dose range finding study. Six dose groups, two comprising of 1 male and 1 female, one comprising of one male and three groups comprising of 3 males and 3 females received a single dose of [redacted] The study duration per dosing was one to three days. During this period mortality and physical condition were recorded at least once a day.

6.7.2. Micronucleus main test

At least five male and five female mice were used per sampling time in each treatment group. The animals were dosed once and sampled according to the following scheme.

Treatment	Dose (mg/kg body weight)	Sampling time (hours)	Group
Vehicle (corn oil)	-	24	A
[redacted] [1)]	15	24, 48	B, C
[redacted]	7.5	24	D
[redacted]	3.8	24	E
Cyclophosphamide	50	48	F

[1)] Five additional animals were used to correct for possible deaths.

6.7.3. Isolation of bone marrow

Bone marrow of the groups treated with [redacted] was sampled 24 or 48 hours after dosing. Bone marrow of the negative control group was isolated 24 hours after dosing and bone marrow of the positive control group was isolated 48 hours after dosing. The animals were sacrificed by cervical dislocation. Both femurs were removed and freed of blood and muscles. Both ends of the bone were shortened until a small opening to the marrow canal became visible. The bone was flushed with approximately 2 ml of fetal calf serum (Invitrogen). The cell suspension was collected and centrifuged at 1000 rpm (approximately 100 g) for 5 min.

6.7.4. Preparation of bone marrow smears

The supernatant was removed with a Pasteur pipette. A drop of serum was left on the pellet. The cells in the sediment were carefully mixed with the serum by aspiration with the remaining serum. A drop of the cell suspension was placed on the end of a slide, which was previously cleaned (24 h immersed in a 1:1 mixture of 96% (v/v) ethanol/ether (Merck, Darmstadt, Germany) and cleaned with a tissue) and marked (with the NOTOX study identification number and the animal number). The drop was spread by moving a clean slide with round-whetted sides at an angle of approximately 45° over the slide with the drop of bone marrow suspension. The preparations were air-dried, fixed for 5 min in 100% methanol (Merck) and air-dried overnight. Two slides were prepared per animal.

6.7.5. Staining of the bone marrow smears

The slides were automatically stained using the "Wright-stain-procedure" in an "Ames" HEMA-tek slide stainer (Miles, Bayer Nederland B.V.). The dry slides were dipped in xylene (Klinipath, Duiven, The Netherlands) before they were embedded in MicroMount (Klinipath) and mounted with a coverslip.

6.7.6. Analysis of the bone marrow smears for micronuclei

All slides were randomly coded before examination. An adhesive label with NOTOX study identification number and code was stuck over the marked slide. At first the slides were screened at a magnification of 100x for regions of suitable technical quality, i.e. where the cells were well spread, undamaged and well stained. Slides were scored at a magnification of 1000x. The number of micronucleated polychromatic erythrocytes was counted in 2000 polychromatic erythrocytes. The ratio of polychromatic to normochromatic erythrocytes was determined by counting and differentiating the first 1000 erythrocytes at the same time. Micronuclei were only counted in polychromatic erythrocytes. Averages and standard deviations were calculated.

6.8. Electronic data capture

Observations/measurements in the study were recorded electronically using the following programme: REES version 1.5 (REES scientific, Trenton, NJ, USA): Environmental monitoring.

6.9. Interpretation

6.9.1. Acceptability of the assay

A micronucleus test is considered acceptable if it meets the following criteria:

a) The positive control substance induced a statistically significant (Wilcoxon Rank Sum Test, one-sided, $p < 0.05$) increase in the frequency of micronucleated polychromatic erythrocytes.
b) The incidence of micronucleated polychromatic erythrocytes in the control animals should reasonably be within the laboratory historical control data range (mean ± three times the standard deviation): Males: 1.3‰ ± 4.3‰ indicated are means for n=251. Females: 1.4‰ ± 3.4‰ indicated are means for n=180).

6.9.2. Data evaluation and statistical procedures

Equivocal results should be clarified by further testing using modification of experimental conditions.

A test substance is considered positive in the micronucleus test if:

- It induced a biologically as well as a statistically significant (Wilcoxon Rank Sum Test, one-sided, $p < 0.05$) increase in the frequency of micronucleated polychromatic erythrocytes (at any dose or at any sampling time) in the combined data for both sexes or in the data for male or female groups separately.

A test substance is considered negative in the micronucleus test if:
- None of the tested concentrations or sampling times showed a statistically significant (Wilcoxon Rank Sum Test, one-sided, $p < 0.05$) increase in the incidence of micronucleated polychromatic erythrocytes either in the combined data for both sexes or in the data for male or female groups separately.

The preceding criteria are not absolute and other modifying factors may enter into the final evaluation decision.

6.10. List of deviations

6.10.1. List of protocol deviations

1. In the dose range finding study the dose level of group B was 517 mg/kg b.w. instead of 500 mg/kg b.w. as stated in protocol amendment 1.
   Evaluation: Due to a calculation error the concentration of [redacted] was too high. This deviation has no effect on the results of the study.

The study integrity was not adversely affected by the deviation.

6.10.2. List of standard operating procedures deviations

Any deviations from standard operating procedures were evaluated and filed in the study file. There were no deviations from standard operating procedures that affected the integrity of the study.

7. RESULTS

7.1. Dose range finding study

In a dose range finding study 23 animals (group A and B: 1 male and 1 female, group C: 1 male, D, E and F: 3 males and 3 females) were dosed intraperitoneally 2000, 517, 100, 20, 10 and 15 mg/kg body weight (groups A, B, C, D, E and F, respectively). The results of this dose range finding study are presented in Table 1.

7.2. Micronucleus main test

Based on the results of the dose range finding study dose levels of 15, 7.5 and 3.8 mg/kg body weight were selected as appropriate doses for the micronucleus main test.

Five male and five female animals were used in each treatment group. Five additional male and female animals, treated with 15 mg/kg body weight [redacted] were used to correct for possible deaths.

The mean body weights per group recorded immediately prior to dosing are presented in Table 2 (Appendix I).

7.2.1. Mortality and systemic toxic signs

The animals of the groups treated with 7.5 and 3.8 mg [redacted] kg body weight and the animals of the negative and positive control groups showed no abnormalities after dosing.

The following clinical observations were made in the groups treated with 15 mg [redacted] kg body weight:

During the first 2 hours after dosing all animals of the group treated with 15 mg/kg body weight were lethargic and had a hunched posture. Eight male and two female animals had a rough coat. Four male and four female animals had their eyes closed.

Within 19 hours after dosing 9 male and 13 female animals had recovered from the treatment. Six male animals still had a rough coat, two of them were still lethargic, had a hunched posture, closed eyes and showed ataxia. Two female animals still had a hunched posture.

Within 43 hours after dosing all animals had recovered from the treatment.

7.2.2. Micronucleated polychromatic erythrocytes

The mean number of micronucleated polychromatic erythrocytes per group and the mean ratio of polychromatic to normochromatic erythrocytes are presented in Table 3 (Appendix I). The individual data are described in Appendix II. The mean number of micronucleated polychromatic erythrocytes scored in [redacted] treated groups were compared with the corresponding solvent control group.

[redacted] induced a statistically significant increase in the mean number of micronucleated polychromatic erythrocytes compared to the corresponding solvent control group in female animals dosed with 7.5 mg/kg b.w. Since the mean number of micronucleated polychromatic erythrocytes per 2000 polychromatic erythrocytes (1.2 ± 1.1) of this group as well as the individual number of micronucleated polychromatic erythrocytes per 2000 polychromatic erythrocytes per animal were well within the historical control data range (0 – 4), the increase was considered not to be biologically relevant.

No increase in the frequency of micronucleated polychromatic erythrocytes, compared to the vehicle treated animals, was observed in the polychromatic erythrocytes of the bone marrow of all other dose groups treated with [redacted]

The incidence of micronucleated polychromatic erythrocytes in the bone marrow of all negative control animals was within the historical solvent control data range.

Cyclophosphamide, the positive control substance, induced a statistically significant increase in the number of micronucleated polychromatic erythrocytes in both sexes (Appendix III). Hence, the acceptability criteria of the test were met.

7.2.3. Ratio polychromatic to normochromatic erythrocytes

The groups that were treated with 7.5 and 3.8 mg [redacted] kg b.w. showed no decrease in the ratio of polychromatic to normochromatic erythrocytes compared to the vehicle controls, which reflects a lack of toxic effects of this compound on the erythropoiesis. In the group treated with 15 mg [redacted] kg b.w., sampled after 48 hours, a decrease in the ratio of polychromatic to normochromatic erythrocytes was observed compared to the vehicle controls. This provides evidence that [redacted] reaches the bone marrow and has a toxic effect on the erythropoiesis following a 48 h exposure. The animals treated with 15 mg [redacted] kg b.w. sampled after 24 hours did not show a decrease in the ratio of polychromatic to normochromatic erythrocytes compared to the vehicle controls.

The groups that were treated with cyclophosphamide showed an expected decrease in the ratio of polychromatic to normochromatic erythrocytes compared to the vehicle controls, demonstrating toxic effects on erythropoiesis.

8. CONCLUSION

It is concluded that this test is valid and that [redacted] is not clastogenic in the micronucleus test under the experimental conditions described in this report.

9. REFERENCES

1. Schmid, W., 1977, The Micronucleus Test, in: Handbook of Mutagenicity Test Procedures, first edition, Eds. B.J. Kilbey et al., Elsevier North-Holland, 235-242.
2. Heddle, J.A. et al., 1984, The Bone Marrow Micronucleus Test, in: Handbook of Mutagenicity Test Procedures, second edition, Eds. B.J. Kilbey et al., Elsevier North-Holland, 441-457.

APPENDIX I TABLES

Table 1 Mortality and systemic toxic signs after treatment with [redacted] in the dose range finding study

Group	Sex	Animal number	Dose mg/kg	Systemic toxic signs* day 1 within 1 hr	2 hrs	3 hrs	day 2 4 hrs after dosing	day 3
A	Male	1	2000	A				
A	Female	2	2000	A				
B	Male	3	517	CFJ	A			
B	Female	4	517	CFJ	A			
C	Male	5	100	F		A		
D	Female	6	20	FJNR			J	B
D	Male	7	20	FJ	FJNR		A	
D	Male	8	20	J	FJN		B	B
D	male	9	20	J	FJNR		A	
D	Female	10	20	B	F		B	B
D	Female	11	20	FJ	FJNR		FGHPRWX[1)]	
E	Male	12	10	B		F	B	B
E	Male	13	10	B		F	B	B
E	Male	14	10	B		F	B	B
E	Female	15	10	B		B	B	B
E	Female	16	10	B		F	B	B
E	Female	17	10	B		B	B	B
F	Male	18	15	FJ	FJNR		B	B
F	Male	19	15	FJ	FJ		B	B
F	Male	20	15	FJ	FJ		B	B
F	Female	21	15	FJR	FJNR		CFJNR	FGHRW[2)]
F	Female	22	15	FJR	FJNR		B	B
F	Female	23	15	FJ	FJ		B	B

[1)] Animal died within 5 hours after observation.
[2)] Animal was sacrificed within 5 minutes after observation.
* Legend 'Mortality and systemic toxic signs':
A = died; B = showed no abnormalities; C = ataxia; F = lethargy; G = no reaction to a stimulus;
H = comatose; J = hunched posture; N = rough coat; P = slow breathing; R = closed eyes;
W = ventral recumbency; X = animal was cold.

Table 2 Mean body weight immediately prior to dosing with [redacted]

Group	Body weight (g) (mean ± S.D.) [1] MALES	FEMALES
A	35.6 ± 0.9	27.8 ± 1.3
B	36.0 ± 1.0	28.6 ± 1.1
C	35.4 ± 1.3	28.6 ± 1.1
D	35.8 ± 1.8	28.2 ± 1.6
E	34.8 ± 1.3	27.2 ± 0.8
F	36.0 ± 2.9	26.6 ± 1.1
Additionally dosed animals	34.0 ± 1.2	29.2 ± 0.8

(1) Five animals per treatment group

Table 3 Mean number of micronucleated polychromatic erythrocytes per 2000 polychromatic erythrocytes and ratio of polychromatic/normochromatic erythrocytes

Group	Treatment	Dose (mg/kg body weight)	Sampling time (hours)	Number of micronucleated polychromatic erythrocytes per 2000 polychromatic erythrocytes (mean ± S.D.) [1]	Ratio polychromatic/ normochromatic erythrocytes (mean ± S.D.) [1]
	MALES				
A	Solvent control	0	24	1.4 ± 1.3	1.08 ± 0.07
B	[redacted]	15	24	1.4 ± 0.9	1.02 ± 0.06
C		15	48	0.4 ± 0.9	0.82 ± 0.17
D		7.5	24	0.4 ± 0.5	1.03 ± 0.08
E		3.8	24	0.2 ± 0.4	0.98 ± 0.06
F	CP	50	48	56.6 ± 36.0 [2]	0.34 ± 0.11
	FEMALES				
A	Solvent control	0	24	0.0 ± 0.0	1.07 ± 0.07
B	[redacted]	15	24	0.8 ± 0.8	1.10 ± 0.23
C		15	48	0.4 ± 0.5	0.81 ± 0.21
D		7.5	24	1.2 ± 1.1 [3]	1.13 ± 0.04
E		3.8	24	0.4 ± 0.9	1.01 ± 0.10
F	CP	50	48	40.6 ± 21.2 [2]	0.35 ± 0.09

Solvent control = corn oil
CP = Cyclophosphamide
(1) Five animals per treatment group
(2) Significantly different from corresponding control group (Wilcoxon Rank Sum Test, P = 0.01).
(3) Significantly different from corresponding control group (Wilcoxon Rank Sum Test, P = 0.05).

APPENDIX II INDIVIDUAL DATA

Individual data (males)
(group A : intraperitoneal injection of corn oil)
(group B & C : intraperitoneal injection of [redacted] at 15 mg/kg body weight)
(group D : intraperitoneal injection of [redacted] at 7.5 mg/kg body weight)
(group E : intraperitoneal injection of [redacted] at 3.8 mg/kg body weight)
(group F : intraperitoneal injection of cyclophosphamide)

Group	Animal number	Number of micronucleated polychromatic erythrocytes per 2000 polychromatic erythrocytes	Ratio polychromatic/normochromatic erythrocytes
A	1	2	1.15
A	2	2	1.00
A	3	0	1.01
A	4	0	1.14
A	5	3	1.11
B	11	1	1.06
B	12	2	0.90
B	13	2	1.05
B	14	0	0.85
B	15	2	1.26
C	21	0	0.68
C	22	2	0.62
C	23	0	0.81
C	24	0	1.00
C	25	0	0.98
D	31	0	1.09
D	32	1	1.07
D	33	0	0.95
D	34	1	0.95
D	35	0	1.10
E	41	1	0.97
E	42	0	1.09
E	43	0	0.94
E	44	0	0.93
E	45	0	0.96
F	51	34	0.43
F	52	33	0.27
F	53	36	0.34
F	54	117	0.19
F	55	63	0.45

APPENDIX II – continued –

Individual data (females)
(group A : intraperitoneal injection of corn oil)
(group B & C : intraperitoneal injection of [redacted] at 15 mg/kg body weight)
(group D : intraperitoneal injection of [redacted] at 7.5 mg/kg body weight)
(group E : intraperitoneal injection of [redacted] at 3.8 mg/kg body weight)
(group F : intraperitoneal injection of cyclophosphamide)

Group	Animal number	Number of micronucleated polychromatic erythrocytes per 2000 polychromatic erythrocytes	Ratio polychromatic/normochromatic erythrocytes
A	6	0	1.11
A	7	0	1.16
A	8	0	0.98
A	9	0	1.05
A	10	0	1.04
B	16	0	1.08
B	17	1	1.38
B	18	2	0.80
B	19	0	0.98
B	20	1	1.26
C	26	1	0.78
C	27	1	0.77
C	28	0	0.66
C	29	0	0.69
C	30	0	1.17
D	36	1	1.19
D	37	1	1.07
D	38	0	1.11
D	39	1	1.13
D	40	3	1.15
E	46	2	0.97
E	47	0	0.89
E	48	0	0.95
E	49	0	1.15
E	50	0	1.07
F	56	27	0.32
F	57	78	0.32
F	58	31	0.50
F	59	30	0.26
F	60	37	0.34

APPENDIX III STATISTICS

Wilcoxon Rank Sum Test.

Number of micronucleated polychromatic erythrocytes per 2000 polychromatic erythrocytes; treatment/control comparison.

Group	Treatment	Dose mg/kg body weight	Sex	P-value (one-sided)	Decision at 95% confidence level
D	[redacted]	7.5	females	= 0.05	significant
F	cyclophosphamide	50	males	= 0.01	significant
F	cyclophosphamide	50	females	= 0.01	significant